Membrane Protein Structure

A Role for The APS

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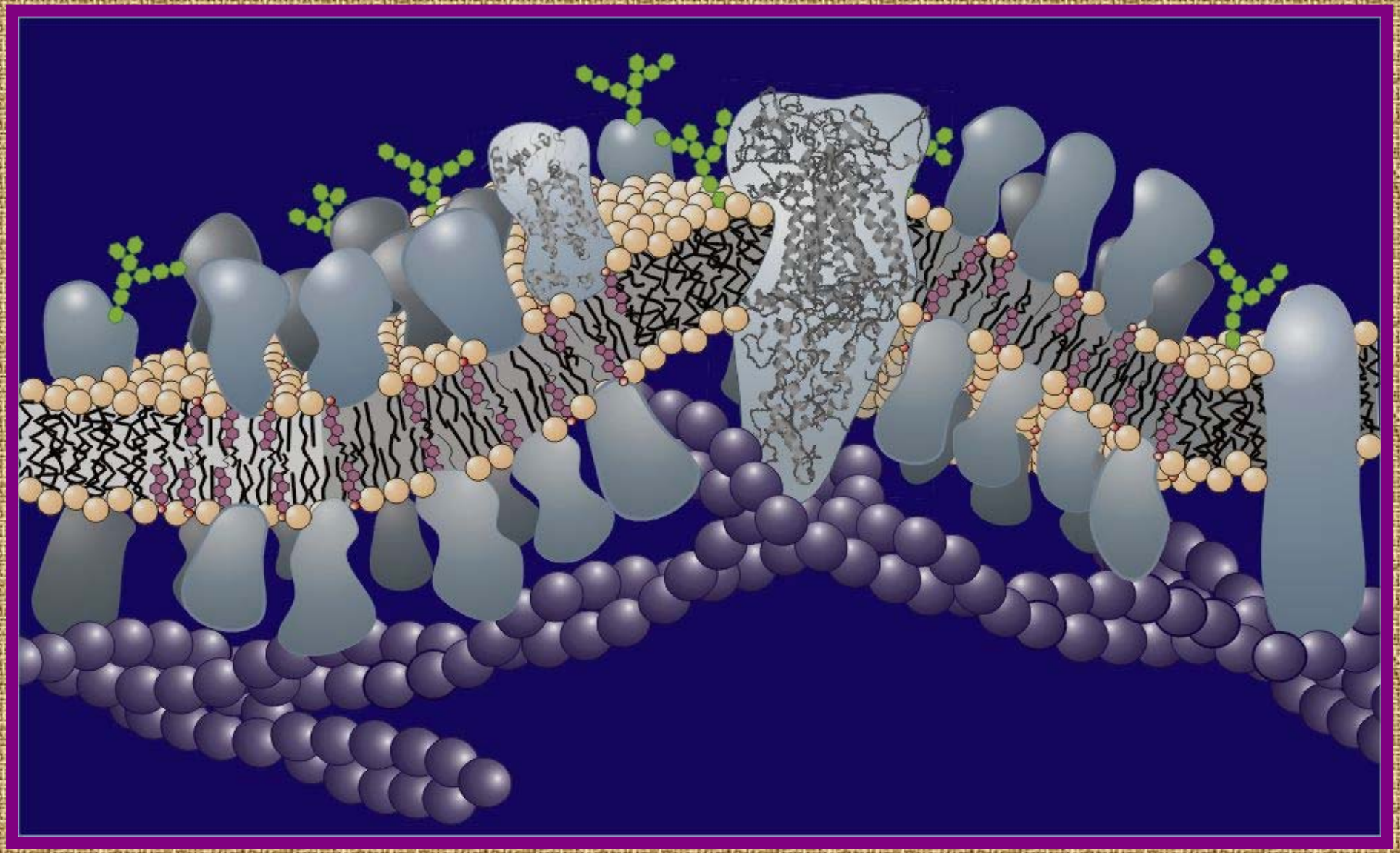
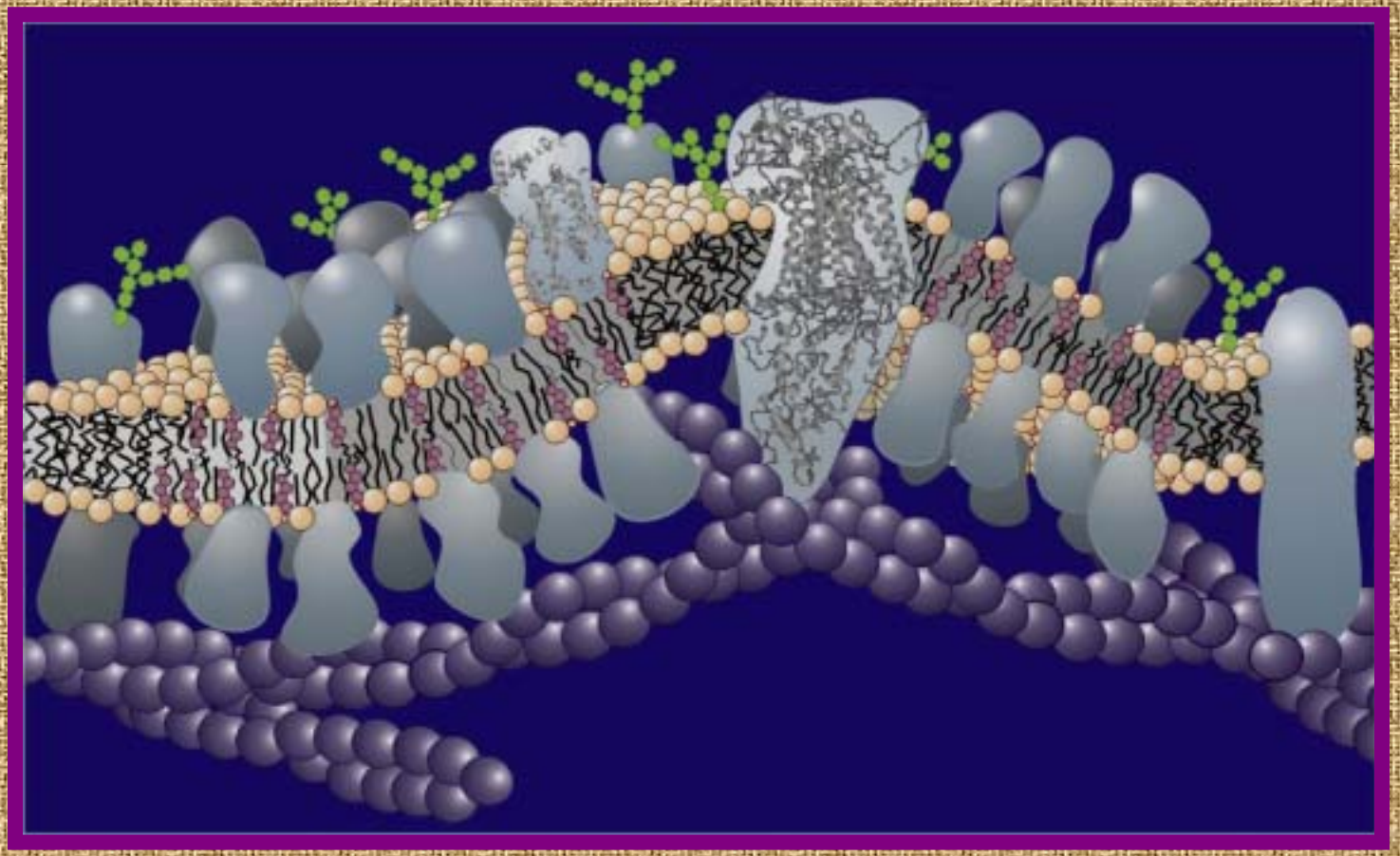
APS Workshop on Membrane Science, ANL. August 2004

O v e r v i e w

- **Membranes**
- **Structure - Function**
- **Bottleneck #1: Production**
- **Bottleneck #2: Crystallization**
- ***In meso* Robot**
- **APS**





Functions

Health significance: senses (sight, touch,hearing, smell, taste)

Aberrations: senses, cystic fibrosis, GPCRs, drug delivery, drug sensitivity/resistance, trafficking, Parkinson's, pain.

Overview

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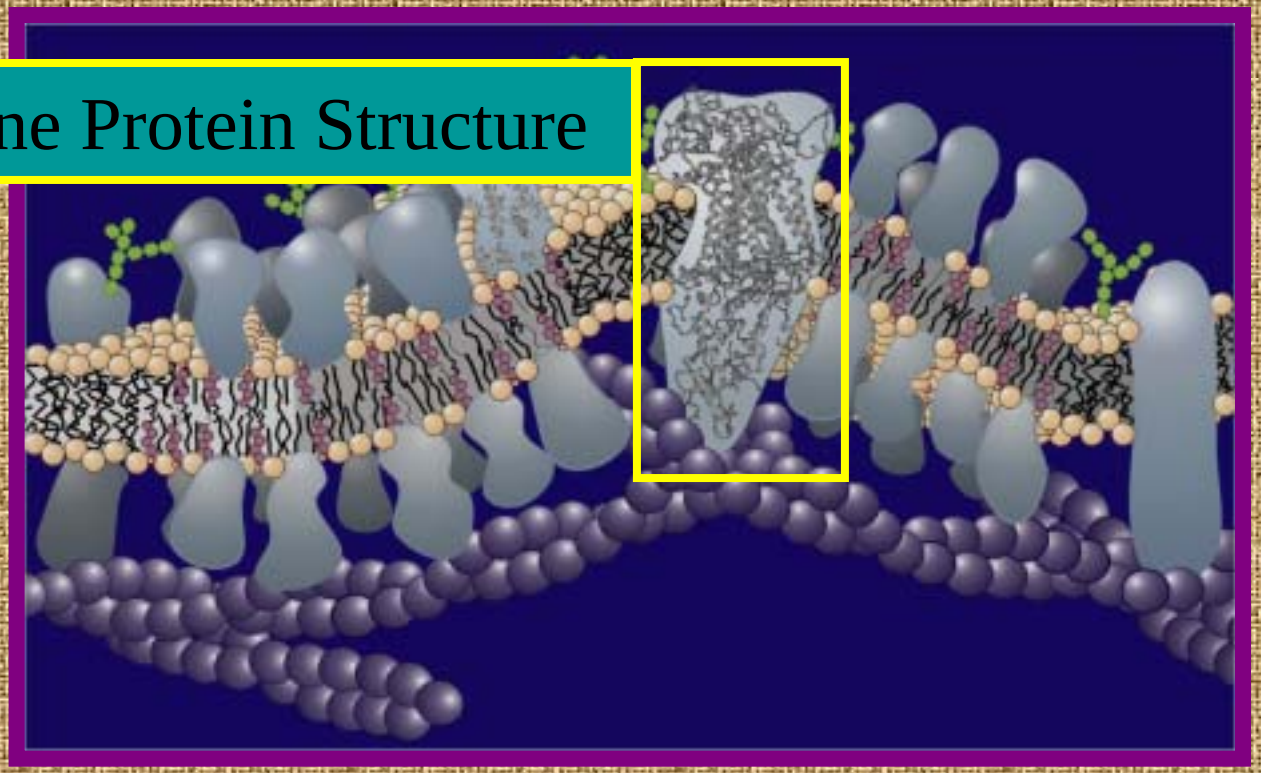
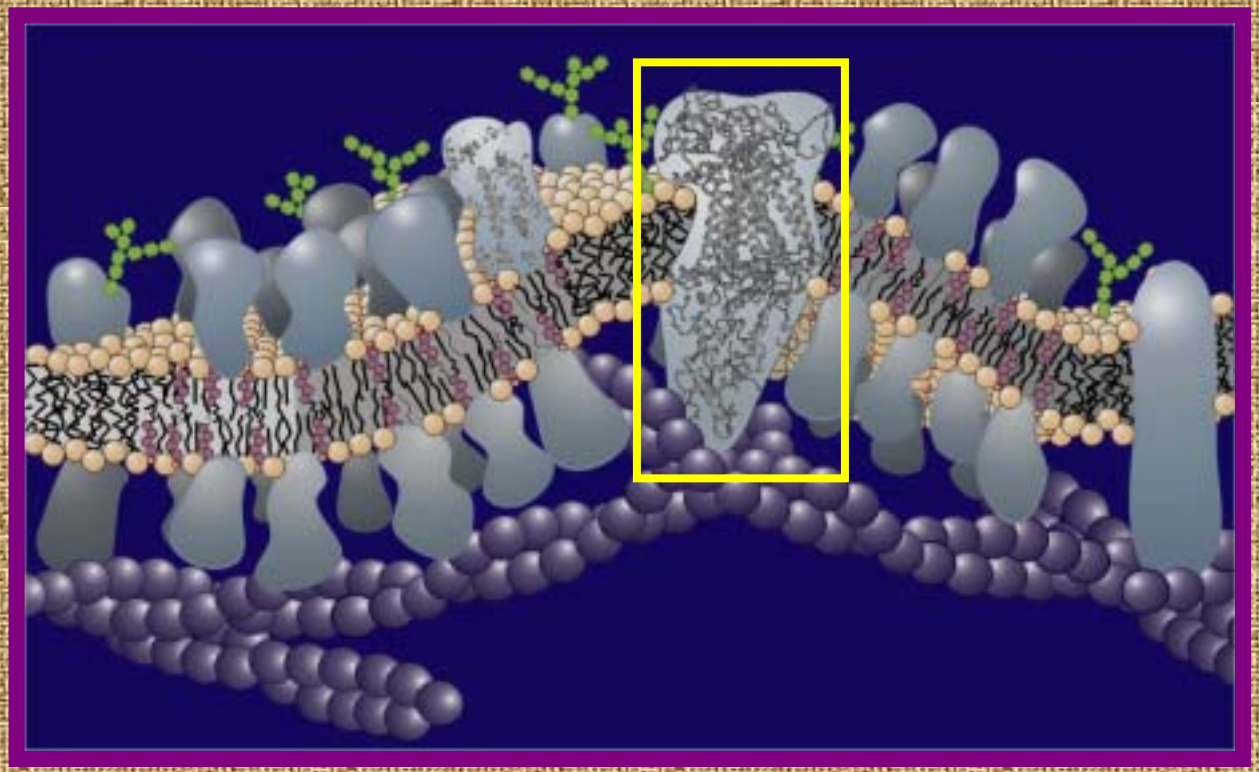
Membrane Protein Structure



Grand Challenge: ***structure dictates function***

Basic knowledge and understanding
Rational design and exploitation
Prevention, treatment, repair

APS contribution: structure via MX



NIH. Human genome project. Structural genomics

'Challenging Proteins' - membrane proteins, complexes and human proteins

Protein Structure Initiative-2: 200 structures/yr @ $50k/structure

Systematic studies to miniaturize, automate, HTP, reduce cost.

APS must prepare for the onslaught.

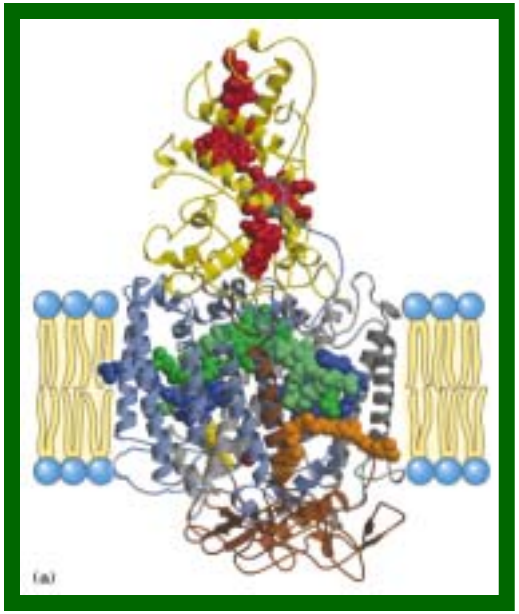
Beam time needs $\propto$ (# samples / useful dataset) (etc.)

Low *versus* high hanging fruit

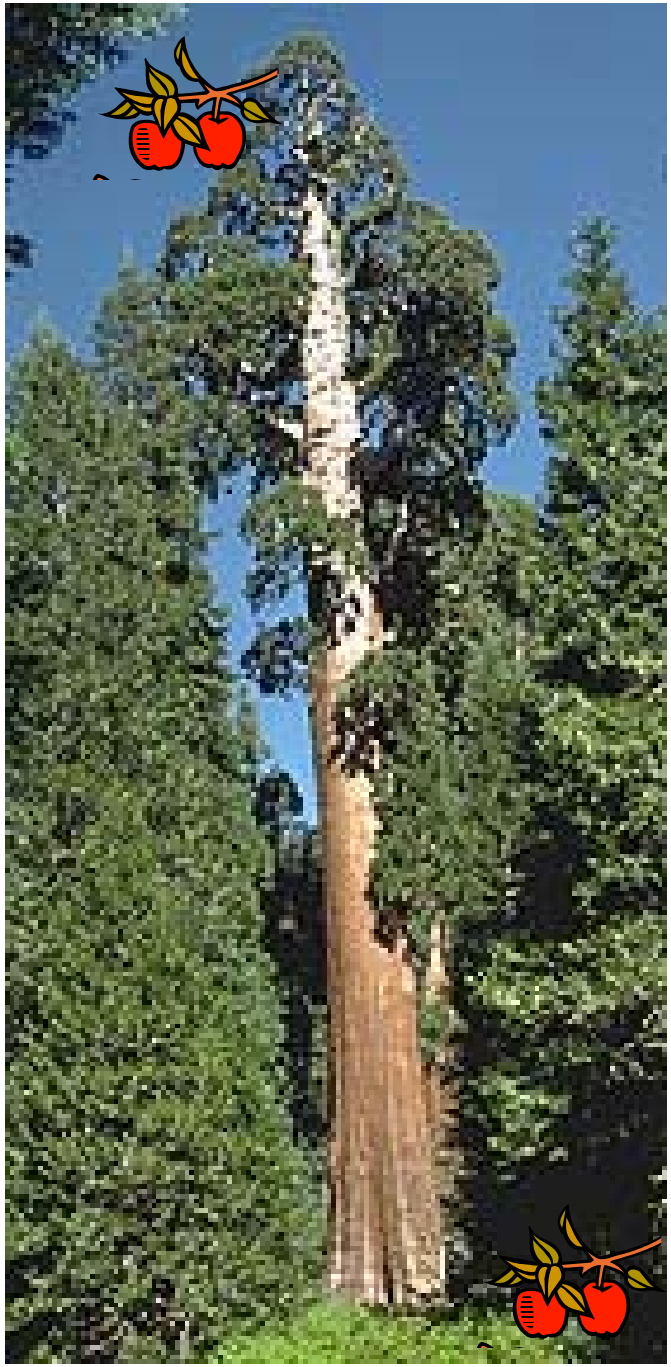
Low *versus* high hanging fruit

Membrane Proteins represent
The Giant Sequoia

- Amphipathic
- Flexible – transport
- Low-abundance



Lots …..



The Numbers Game

27 % of ORF in sequenced genomes code for integral membrane proteins

Human Genome: 30,000 - 50,000

Numbers are large

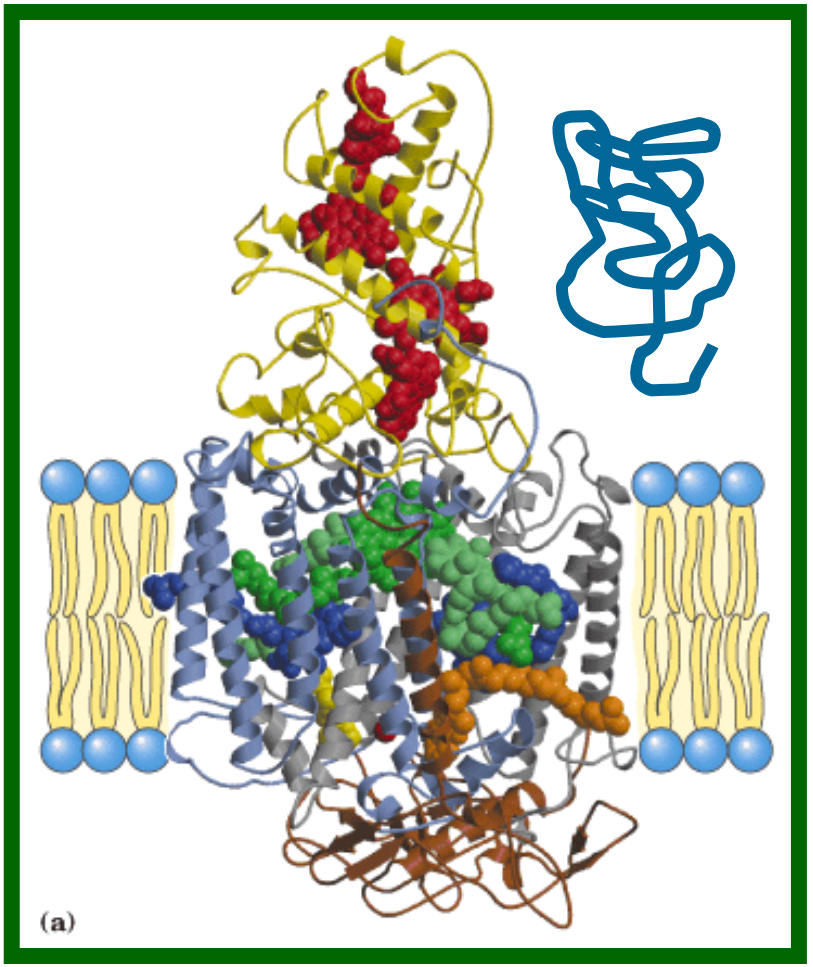
Head start: ~25 unique known structures
- PDB

To work down the deficit ….

Diffraction-Quality Crystals

2 Major Challenges

Protein **Crystal**



Produce

Solubilize / purify / (refold / stabilize / reconstitute)

Crystallize

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Mammalian Protein Production

Yield Pure Homogenous Structure-grade Functional

Natural sources: Muscle, Eye, Heart, RBC, e-Organ

Prokaryotes: *E. coli*, *L. lactis*

Eukaryotes: Yeast: *S. cervicia*, *Pichia*

Mammalian: Lots, HEK293, ±GnT1$^-$, suspension

Complex, expensive, but has what it takes

Cell-free, *In vitro*: *E. coli*, wheat germ

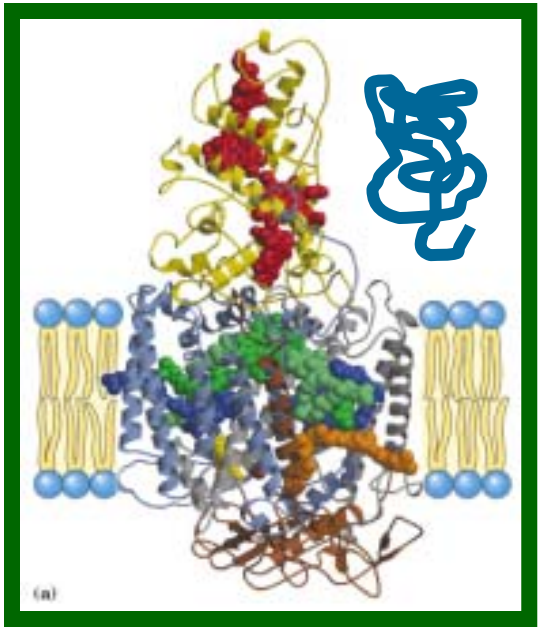
Expensive, advantages, high-risk/payoff

Others: Algae (*chlorella*), caterpillars, Baculovirus/Sf# Insect

Challenges ……

Heterologous Expression (CFTR in *E. coli* or yeast)

Lots but **insoluble**, inclusion bodies, that may or may not **refold**



or

improperly folded in host membrane
non-functional (alien lipid profile)

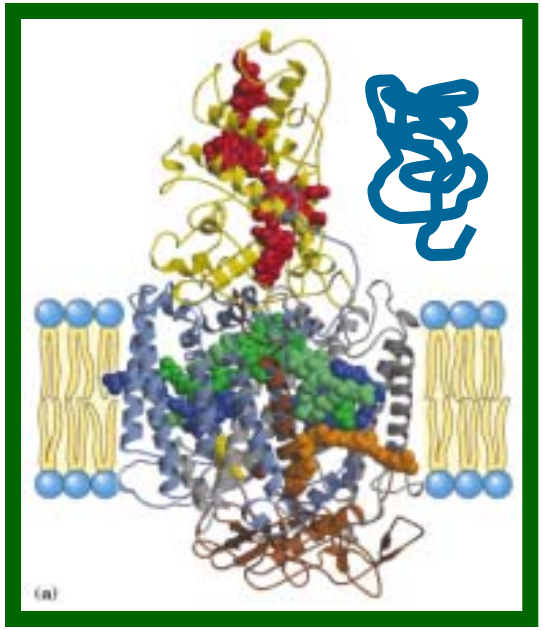
and/or

not **post-translationally modified** correctly (sugar, lipid, clipping, -SS-)

Co-express/Engg chaperones, folding/trafficking enzymes, signal sequences, membrane proliferation, mutate for stability/crystallizability.

Solubilize Purify Refold Stabilize Reconstitute

Typically **detergent**, possibly other **small molecules**, **thousands** to choose from, **no rules**. Use the one in which protein is **stable**. **Screen** for solubilization, stabilization, crystallizability



May need a **mix** of detergents, small molecules, bridging ions

May need **one detergent for purification another for stability/cost,** exchange

Purification Tags for affinity chromatography.
N-, C-, internal, screen. Types His, Flag, fusion MBP, Antibodies
Screen for +/- retention for crystal structure determination

Purify, but not to death !

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Crystallogenesis

Crystallizability a part of **Quality Control** and a **Conduit to Structure**.

Measure of: **yield, purity, structural and chemical homogeneity**

functional ?

– no guarantee!

Diffraction Quality

– **Structure-grade ?**

Methods for Crystallizing Membrane Proteins

- *In surfo*
- *In meso* (*cubo*, vesicle, bicelle)
- Jig - saw
- Bypass
- Ax
- Wetting ?

LIQUID

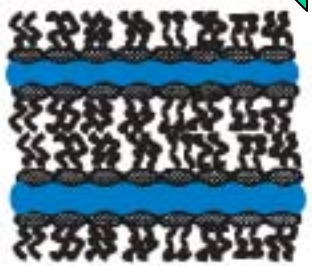
LIQUID CRYSTALLINE

Fluid isotropic
FI

Lamellar crystal
L_c

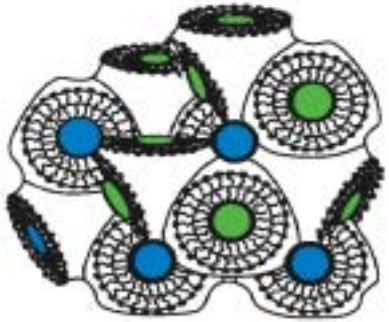
SOLID

Lamellar
liquid crystal
L_α

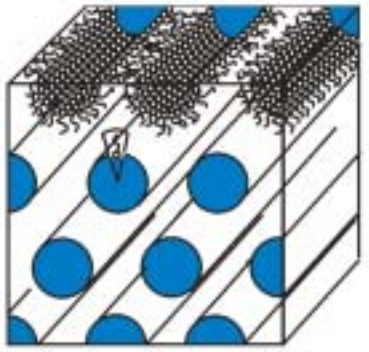


Biomembrane

Cubic
Pn3m

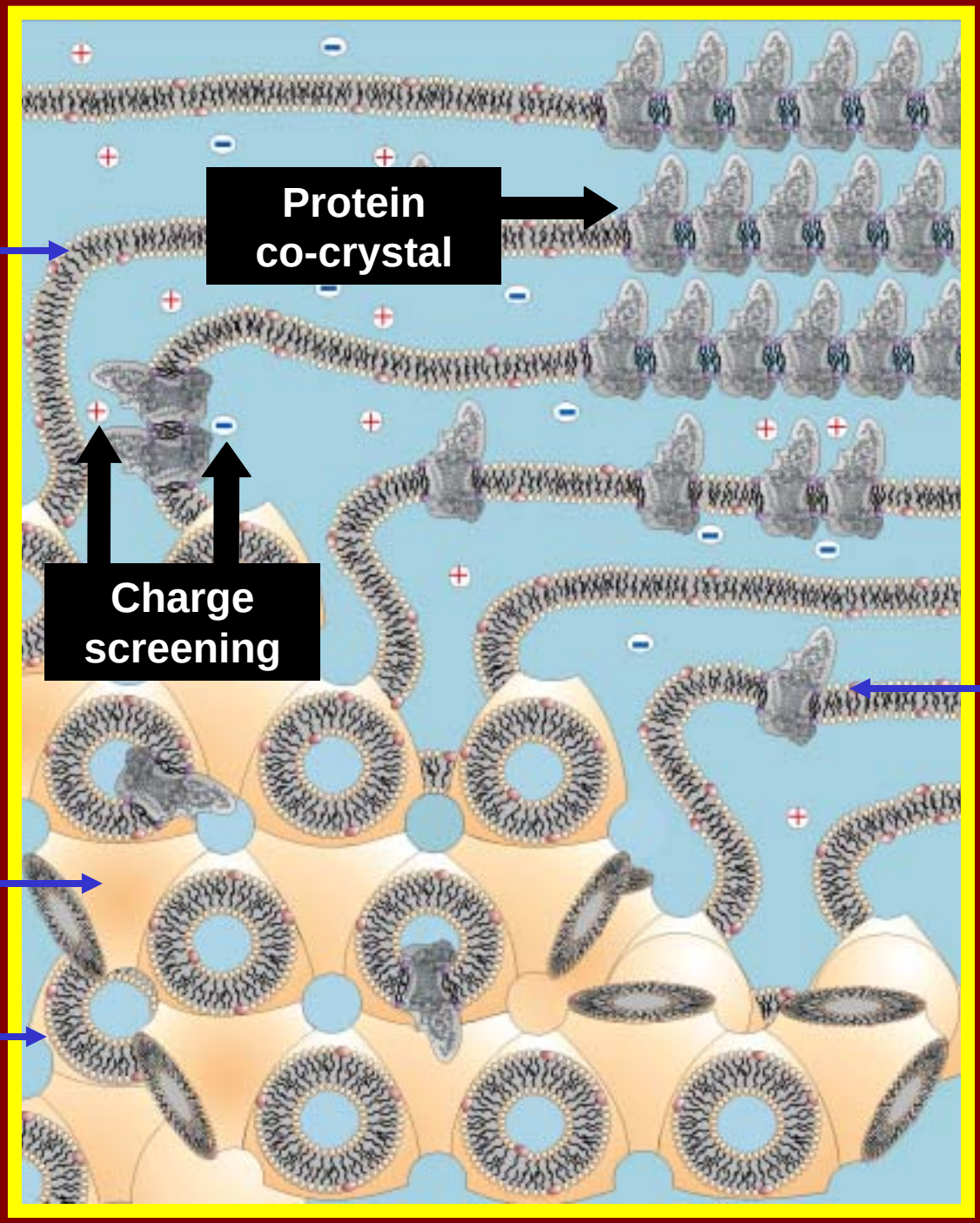


Inverted hexagonal
H_{II}



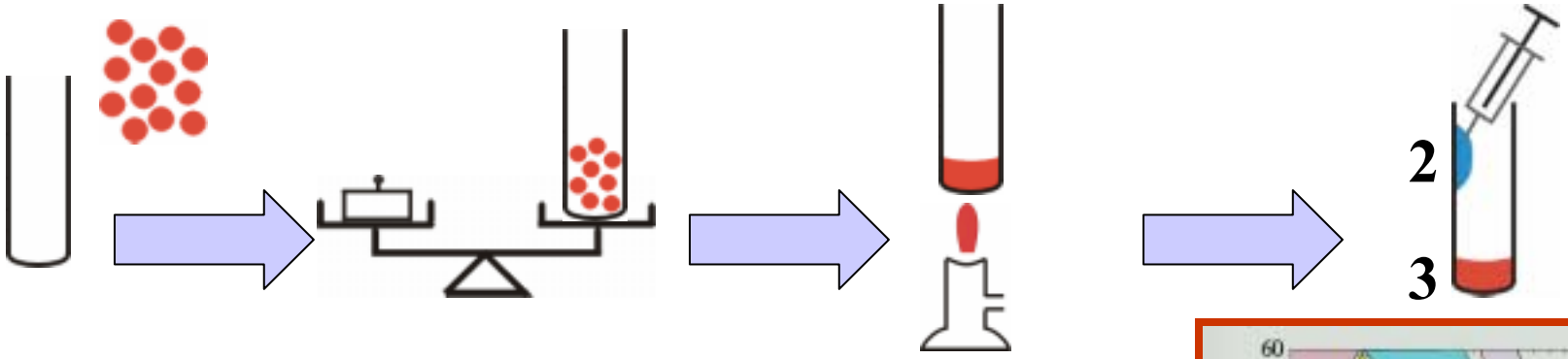
mesophases

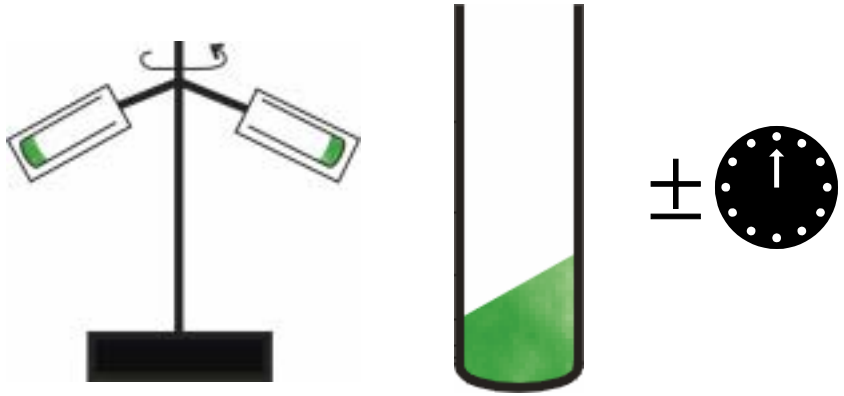

Structure of bR crystallized in lipid cubic phase
Science 277:1676,1997
Vadim Cherezov
20 µm

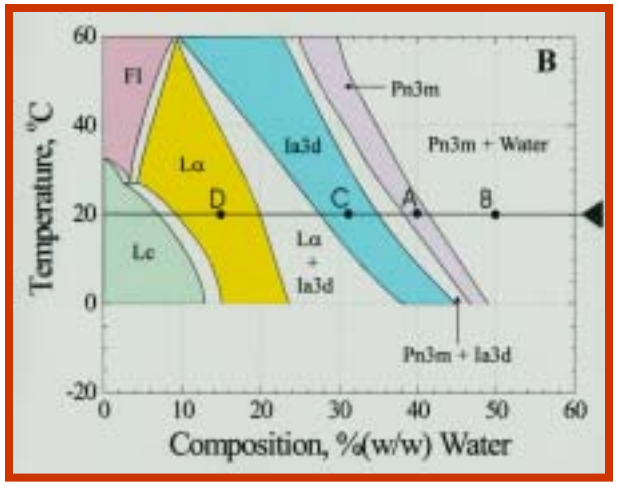


J. Struct. Biol. 142:108-132

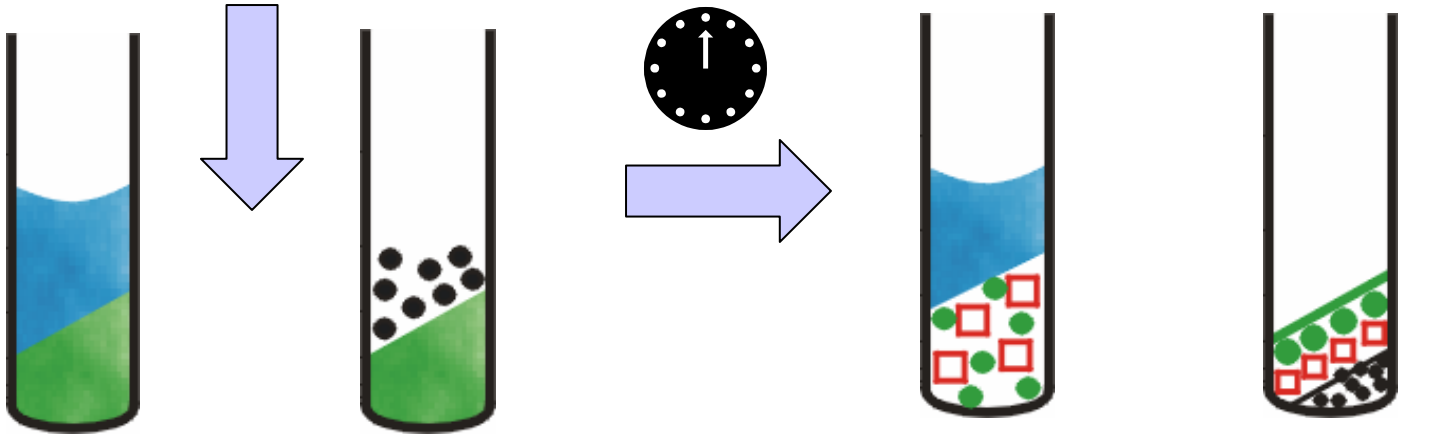
The Original Method







≥ 1 hour per 5 mg Sample



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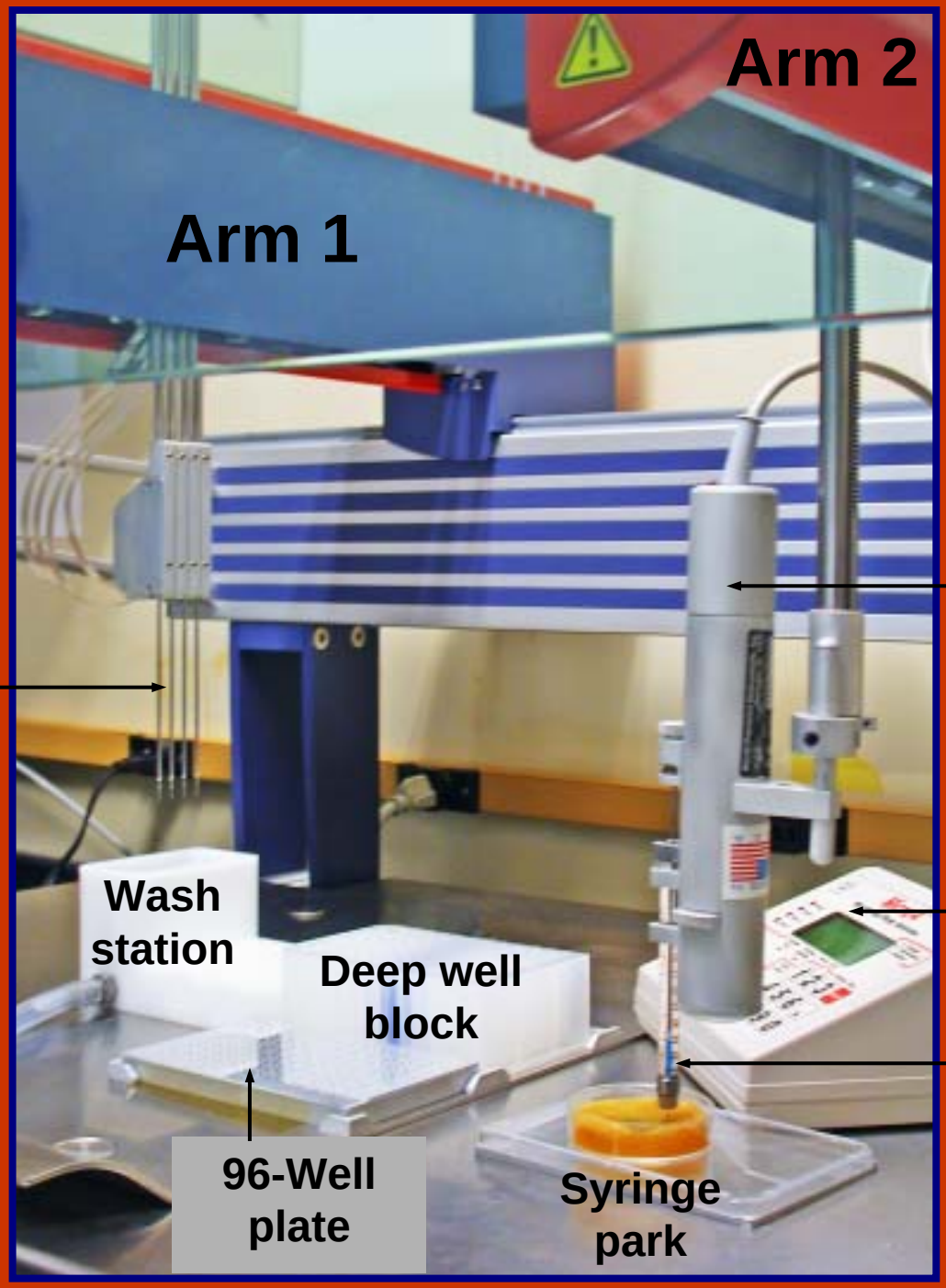
- ***In meso*** **Robot: Miniaturize, Auto, HTP, Cost**
- **APS**

In Meso Robot

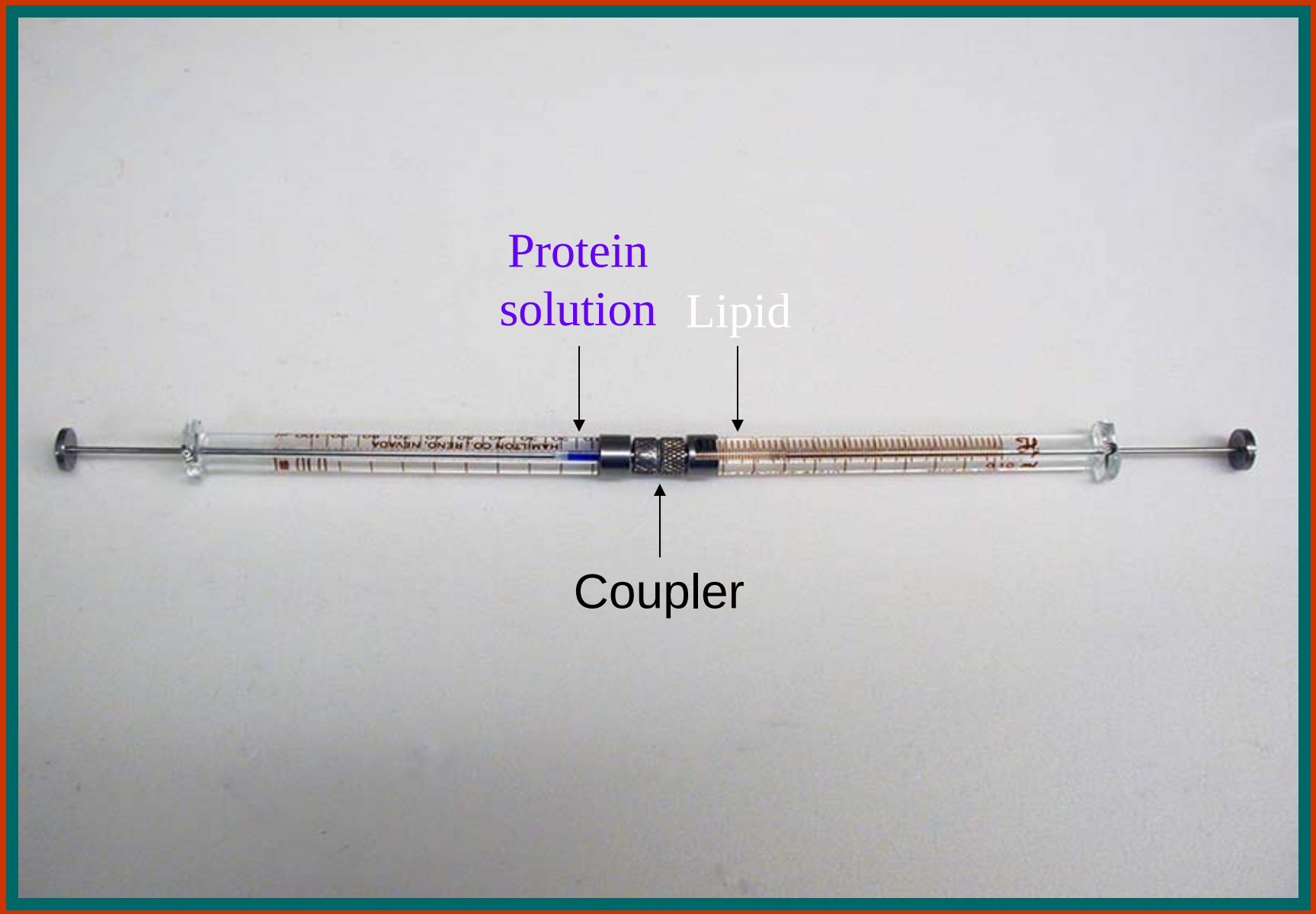
Liquid Handling Robot

Motorized Micro-pump

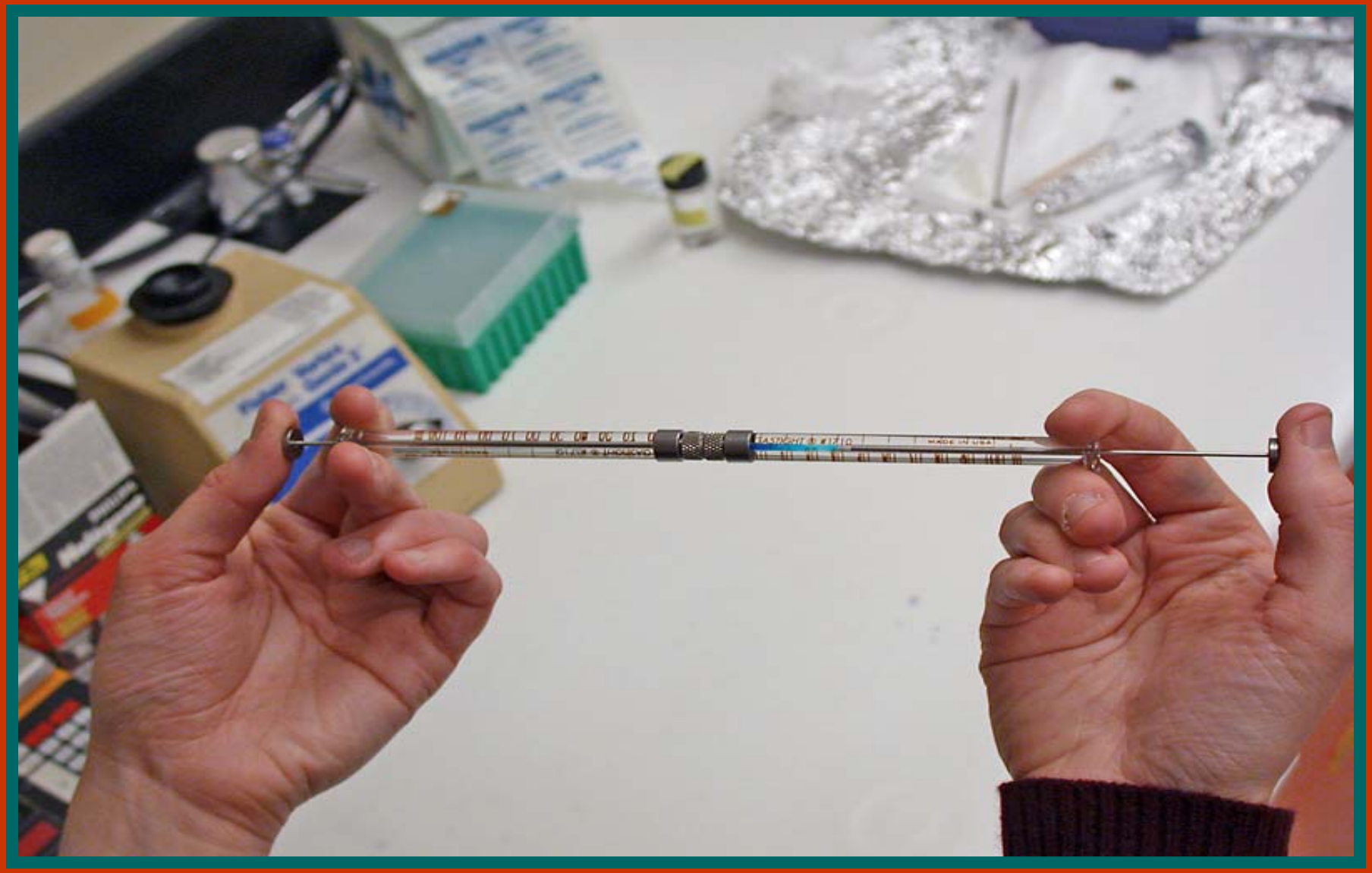


Acta D. In press

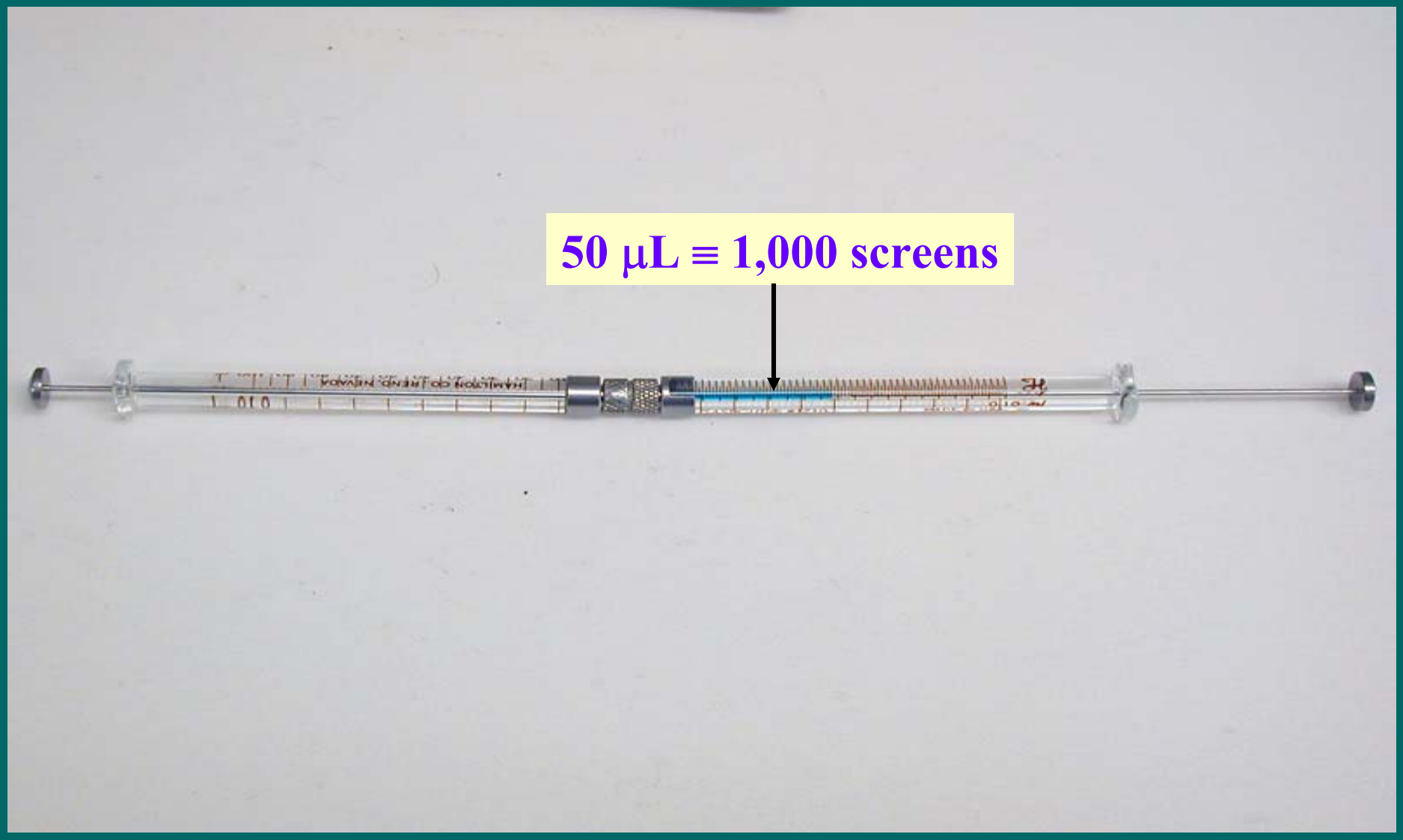
Step-by-Step Use of the *In Meso* Robot



Syringe Mixer containing blue membrane protein solution in the syringe on the left and lipid in syringe on the right. Ratio of lipid to protein solution is ~ 3 / 2 by vol.

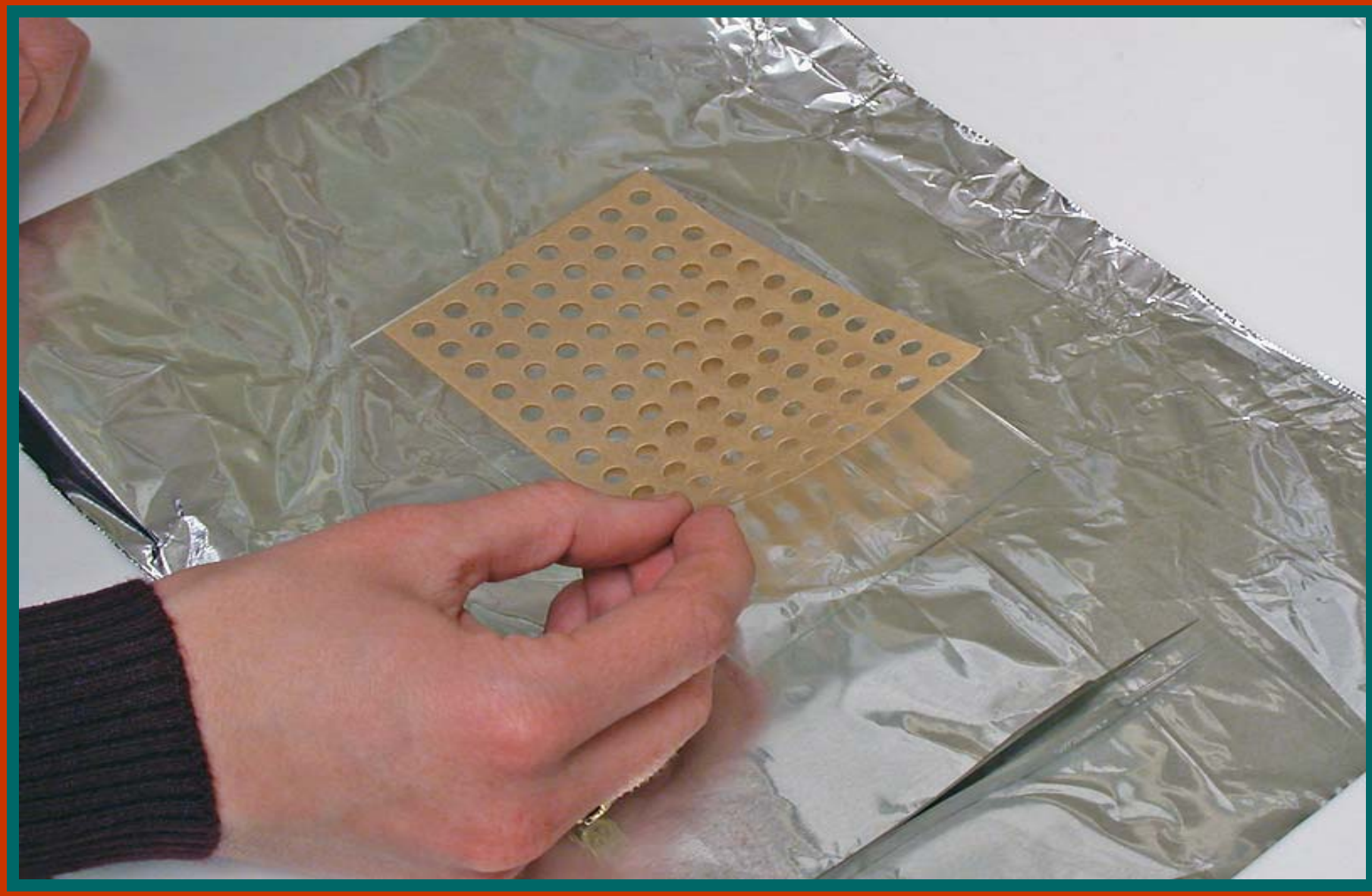


Mechanically mixing the protein solution and lipid together to form the cubic mesophase.



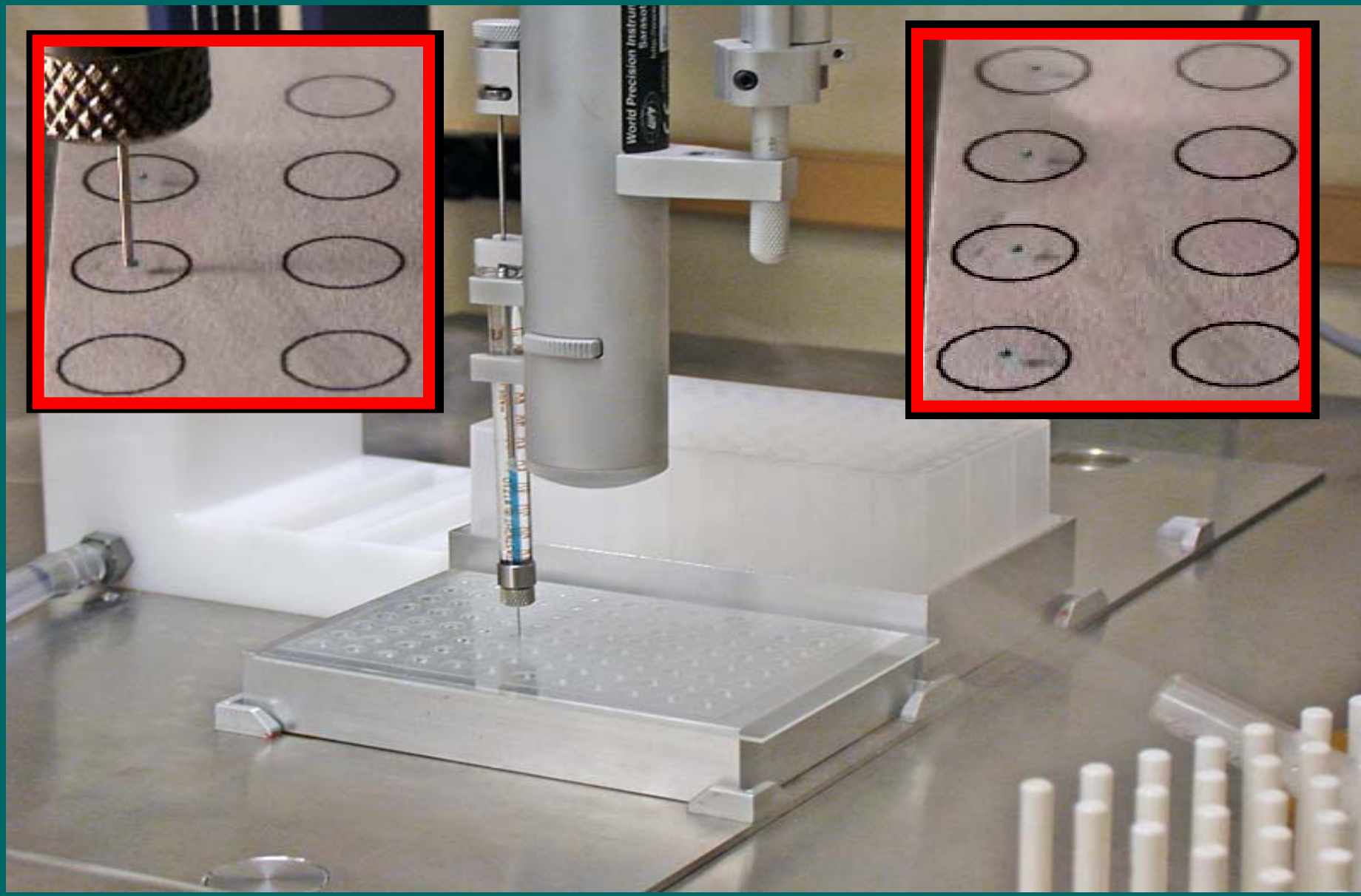
Cubic phase formed after mixing as can be seen in the syringe on the right hand side

Syringe containing protein/lipid dispersion attached to automated pump on robot arm #2. Pump controller is located on the left hand side of picture.

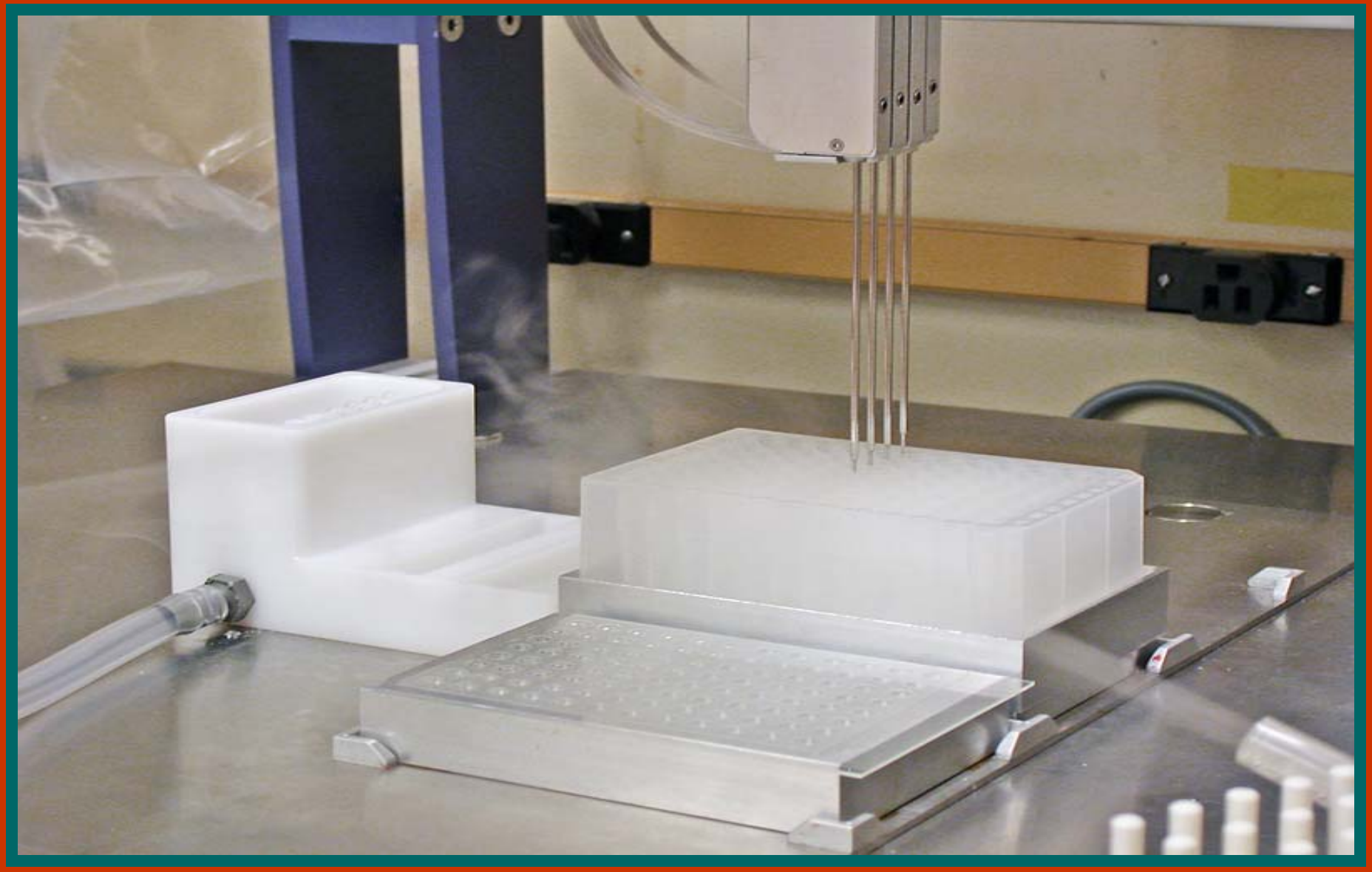


Preparation of 96-well plate: attaching perforated spacer to clean glass slide via adhesive backing

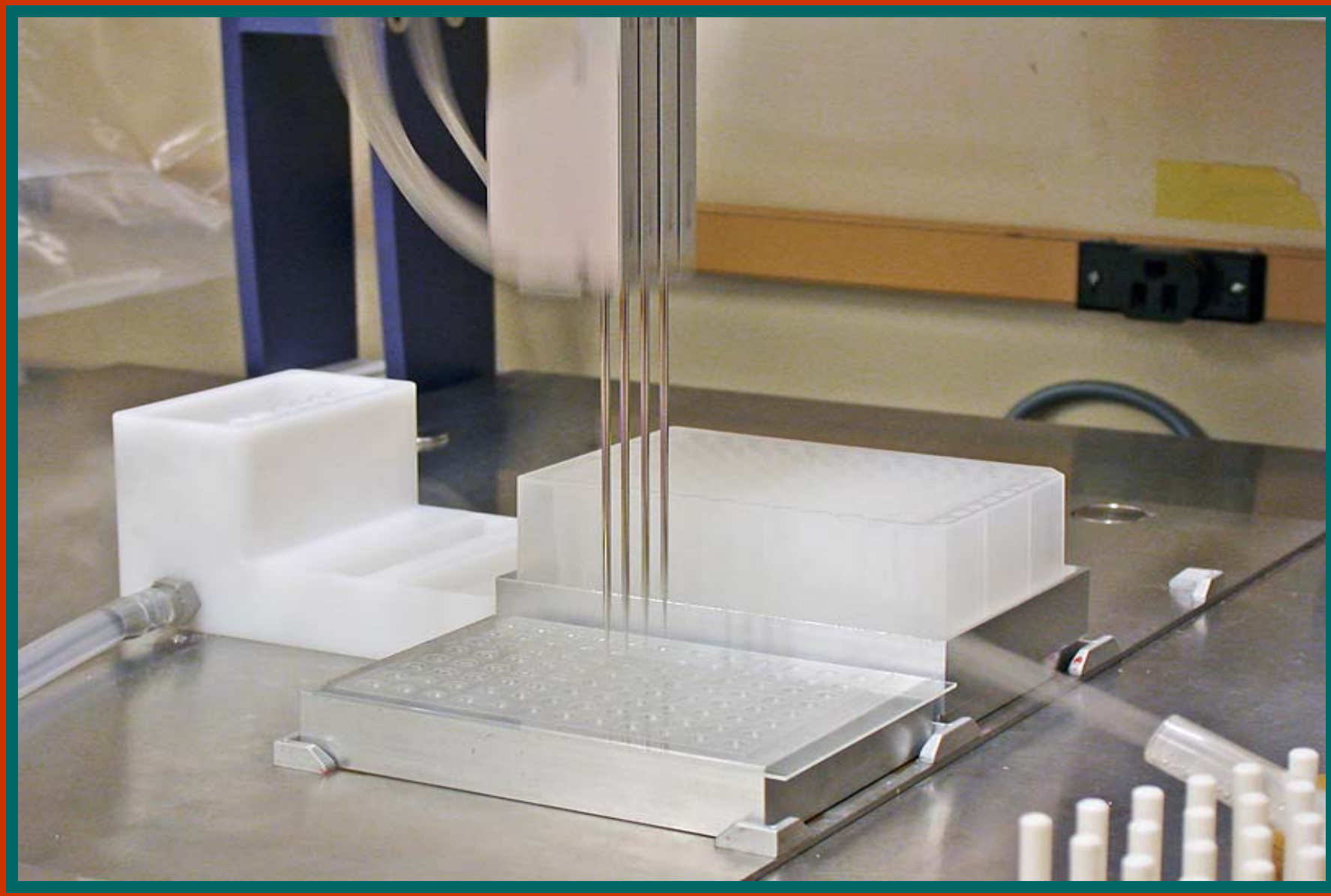
The plate/spacer is placed on the block and aligned to the imprinted template. Precipitant solutions in a 96-well block are placed in the holder behind the plate.



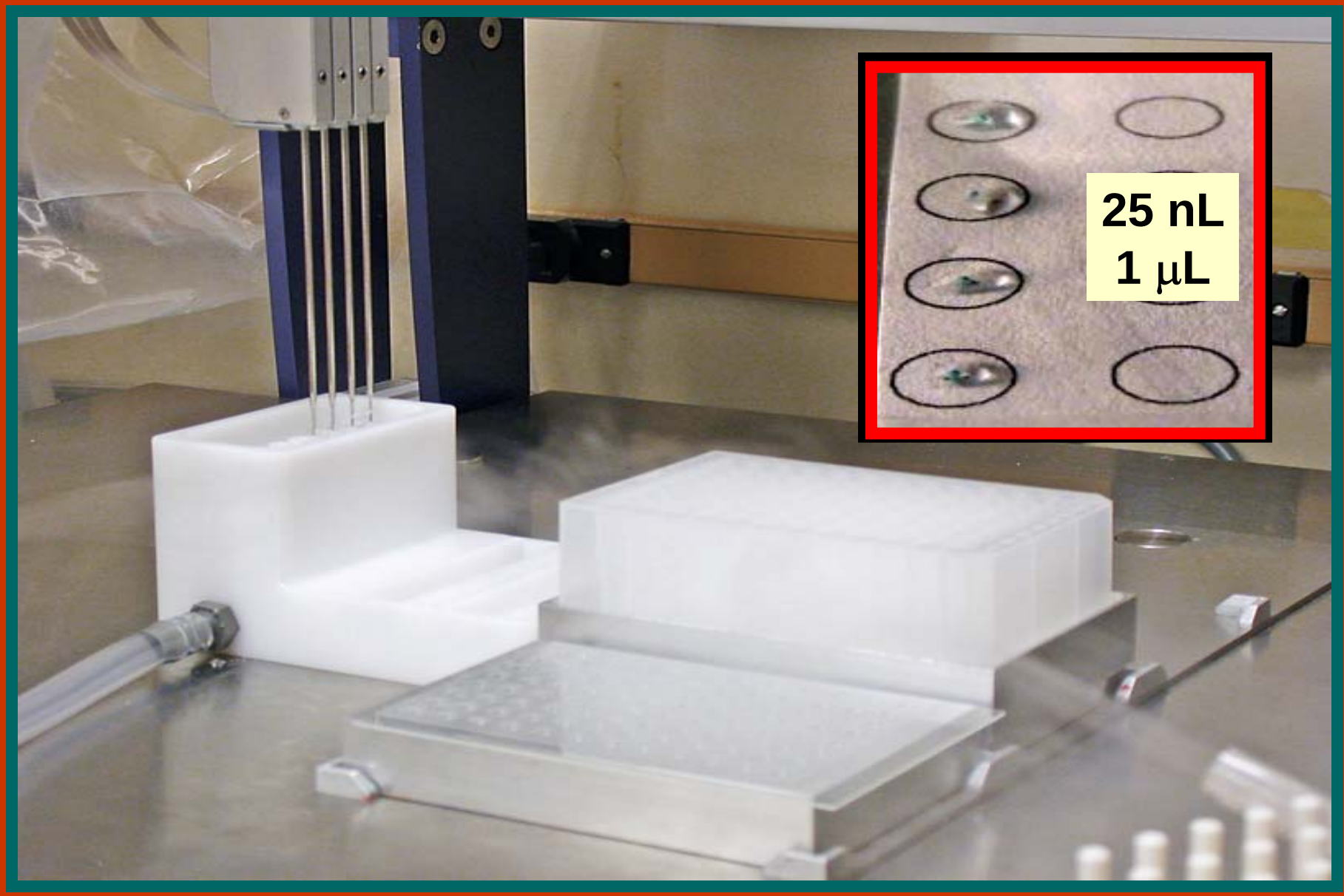
Syringe dispensing 25 nL of cubic phase into centre of well.



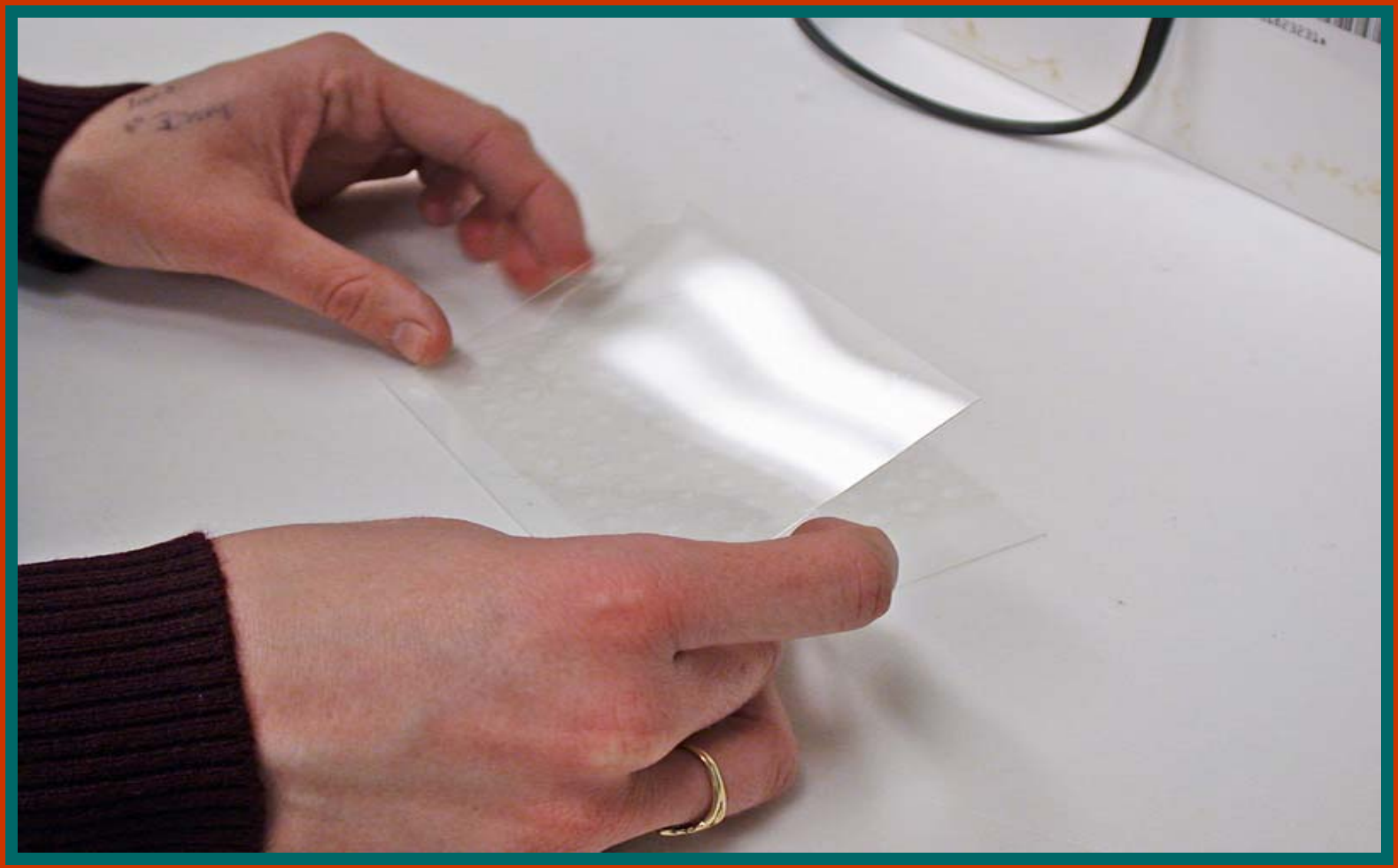
Tips on arm #1 aspirating 4 precipitant solutions from 96-well block.



Dispensing of precipitant solutions on top of cubic phase droplet in centre of well



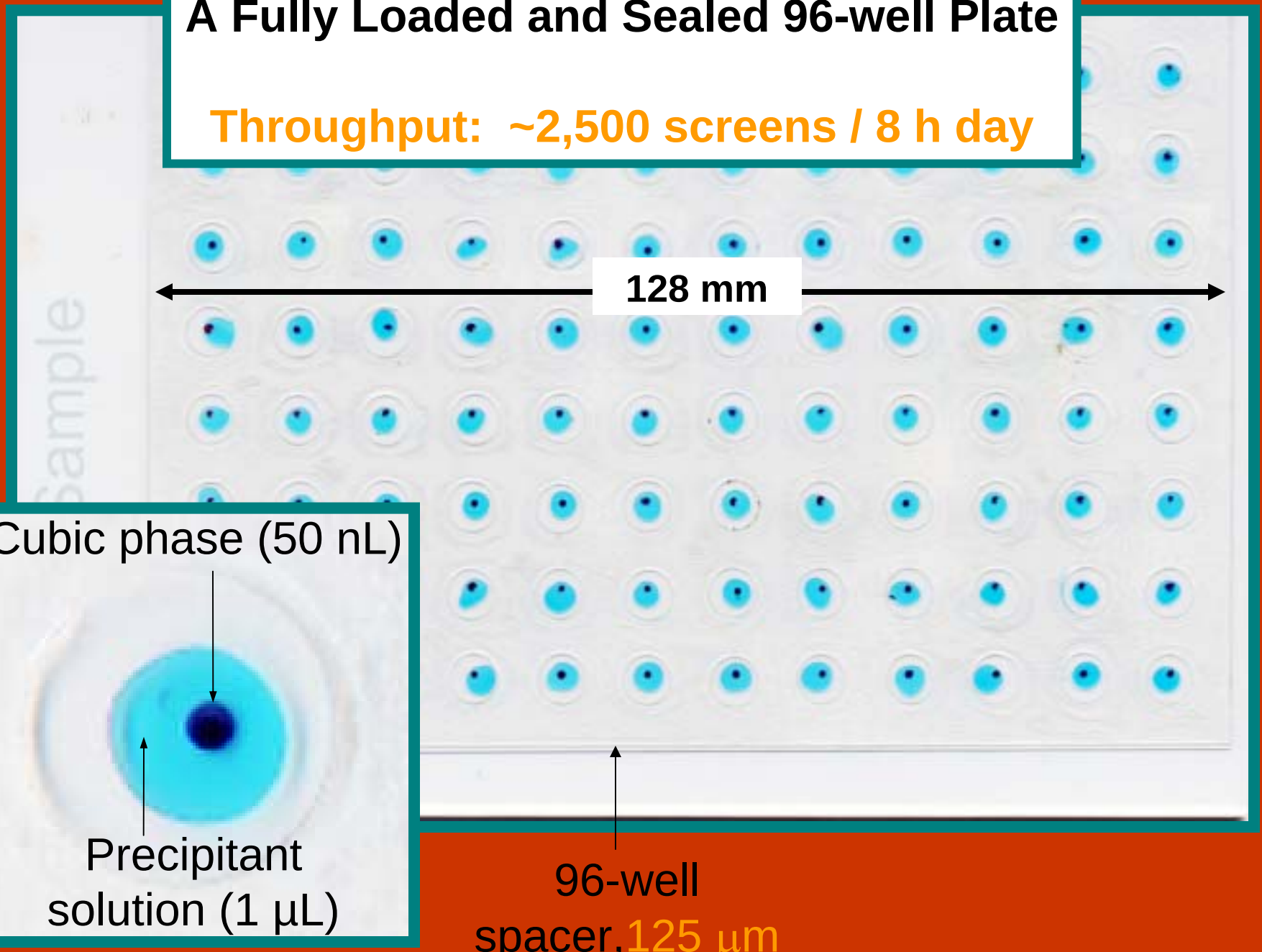
Cleansing tips at wash station



Dispensing complete. Plate is sealed with clean glass coverslip.

A Fully Loaded and Sealed 96-well Plate

Throughput: ~2,500 screens / 8 h day



Manual viewing of wells in 96-well plate through microscope for crystal growth

Imaging robot

Crystals Grown Using The *In Meso* Robot: Versatile

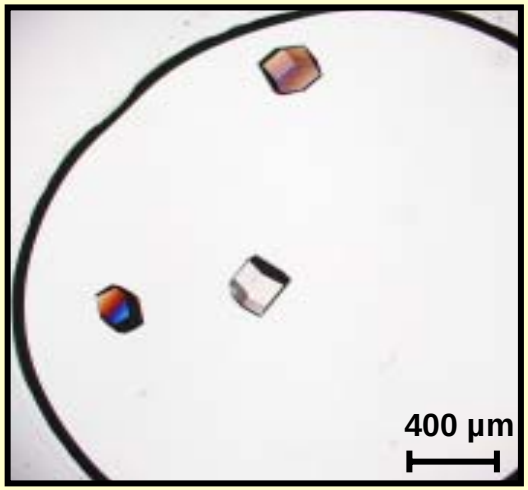
A: In Meso, bR

Plus

Economical

HTP

B: Batch, Lysozyme



C: Bicelle, bR

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How Can APS Help ?

Small crystals, Large unit cells, High solvent content

Radiation-sensitivity

High-throughput robotic screening for diffraction quality

During screening: option to T-anneal, desiccation, etc.

***In situ* screening**

'Fed Ex' service. Remote evaluation of diffraction quality

Direct crystallography. 2 Å lines. S-anomolous

Standardize equipment, software and interfaces

Staffing: est. 5/beam line. BioSync Report '02. **Solved structures**

X-ray scattering: homogeneity, size, shape

Fast/simple access, quick turnaround, pleasant experience

ACKNOWLEDGEMENTS

Collaborators

University of Limerick
The Ohio State University

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O Slattery, Y Zheng

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National Science Foundation
Science Foundation Ireland

References

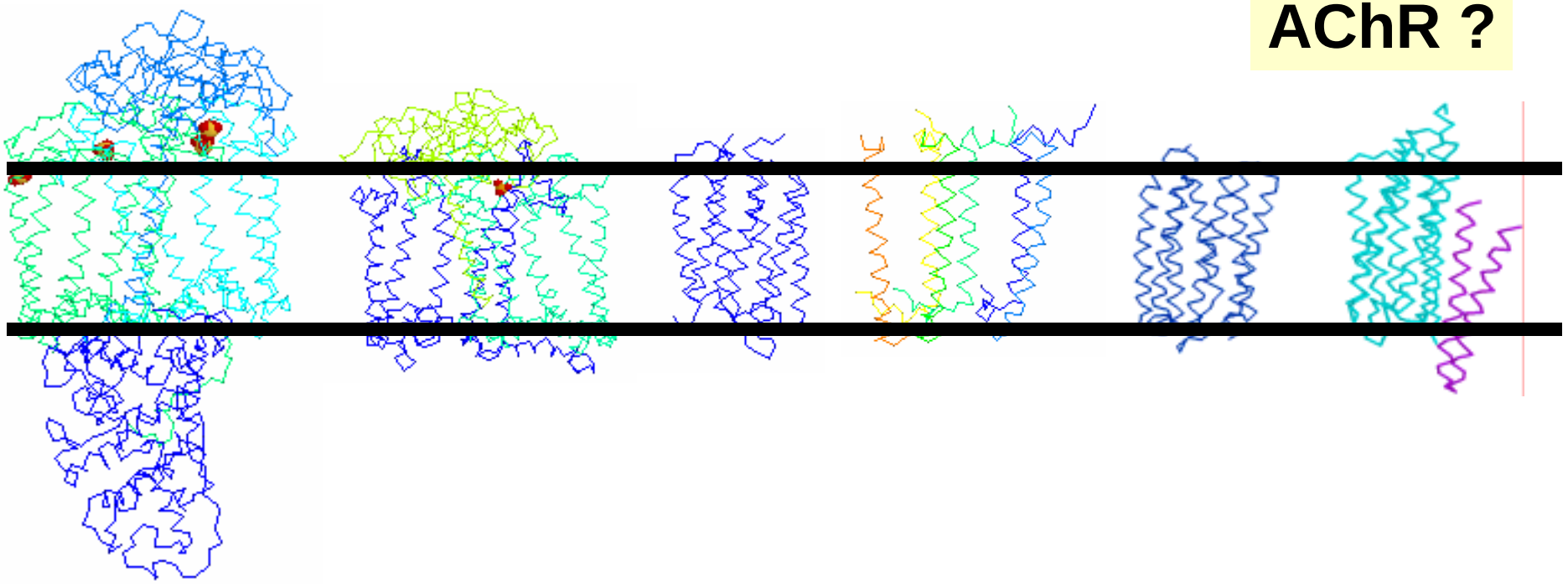
- Acta D. In press
- J. Struct. Biol. 142: 108 - 132.

The *In Meso* Method

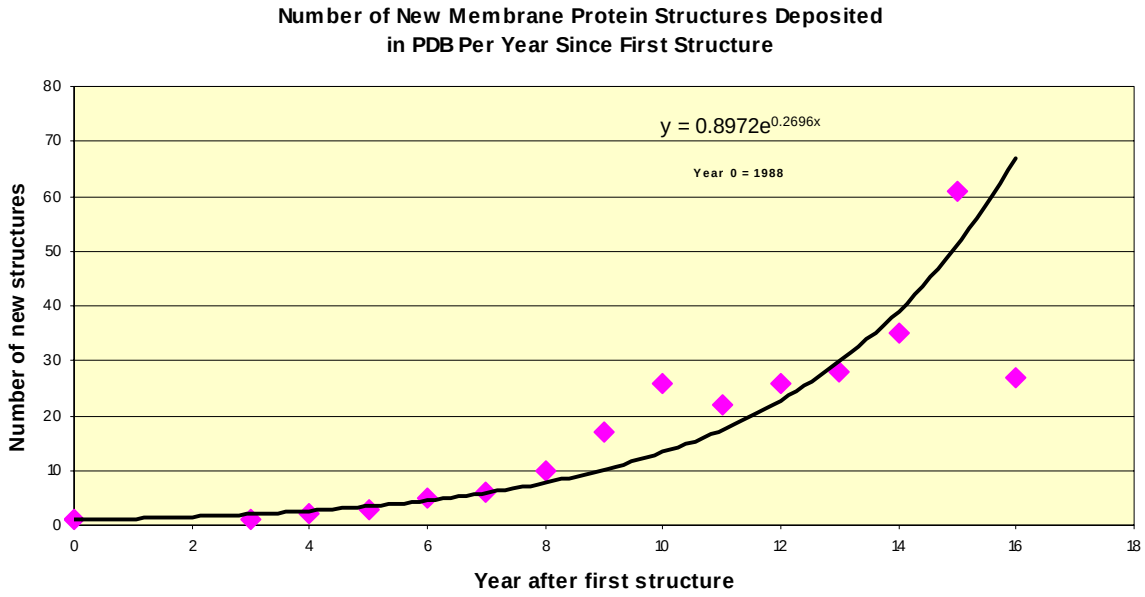
Generality

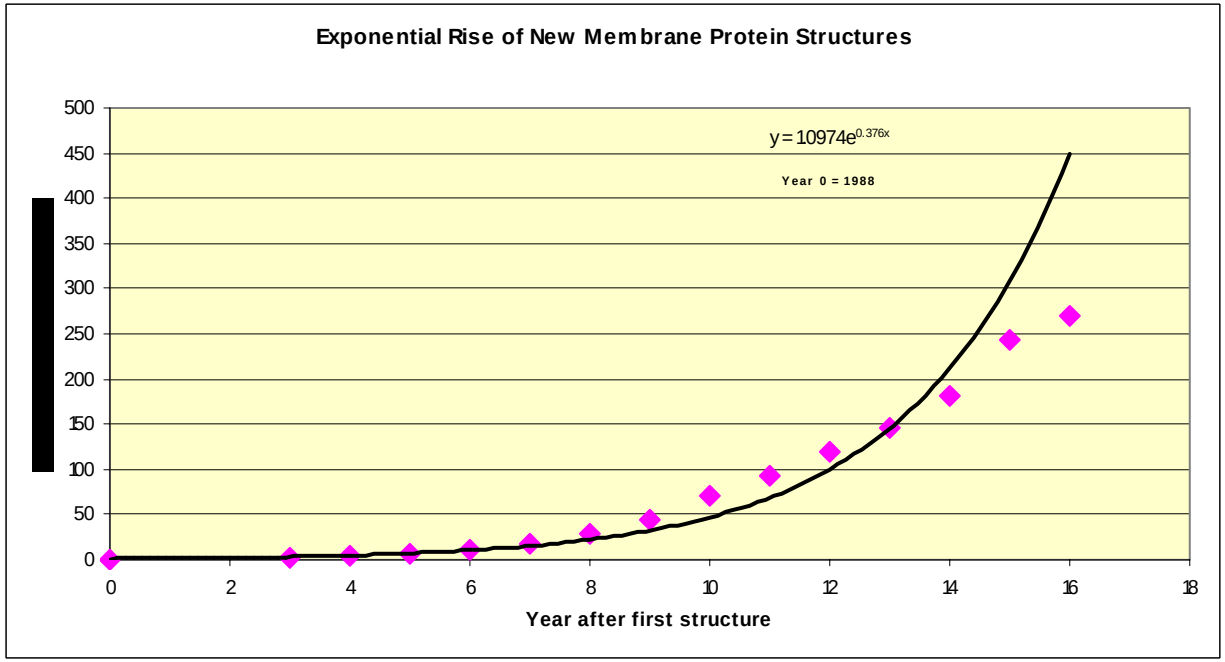
Rxn Center (*Rp. viridis*, *Rb. sphaeroides*), bR, hR, LH2, Sensory Rhodopsin II, SR II/Transducer.

AChR ?



1DXR 1PCR 1C3W 1KZU 1JGJ 1H2S


Number of New Membrane Protein Structures Deposited
in PDB Per Year Since First Structure
y = 0.8972e0.2696x
Year 0 = 1988
Number of new structures
Year after first structure
0
10
20
30
40
50
60
70
80
0
2
4
6
8
10
12
14
16
18


Exponential Rise of New Membrane Protein Structures
y = 10974e0.376x
Year 0 = 1988
Year after first structure
0
50
100
150
200
250
300
350
400
450
500
0
2
4
6
8
10
12
14
16
18